Revista Virtual de Química

Article

Rev. Virtual Quim. **2026**, *18(6)*, 829-840 – http://dx.doi.org/10.21577/1984-6835.20260053

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Analytical Techniques for Monitoring Sodium Metabisulfite in Shrimp: A Mini-Review

Técnicas Analíticas para Monitoramento de Metabissulfito de Sódio em Camarão: Uma Mini-Revisão

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Submitted: May 5th, 2026 – **Accepted:** July 22nd, 2026 – **Published online:** August 4th, 2026

Associate Editor Handled: João Flávio Petruci

Sodium metabisulfite is widely used in the shrimp industry to prevent melanosis and extend product shelf life. However, sulfite residues above regulatory limits can pose risks to consumer health, making the development and application of reliable analytical methods essential for monitoring these residues. This study presents a bibliometric literature review to analyze the evolution of research on methods for determining sulfites in shrimp. The most cited modern analytical techniques included biosensors, electrochemical methods, high-performance liquid chromatography, and ion chromatography. The results showed a continuous growth in scientific production, reflecting increased concern for food safety and the strengthening of international regulations. Although the Monier-Williams method remains the regulatory reference, chromatographic techniques, such as ion chromatography and HPLC, continue to show greater robustness, selectivity, and reliability for confirmatory analyses. In contrast, electrochemical methods stand out for their short response time, reduced reagent consumption, and potential for portable applications and real-time monitoring. Thus, there is a trend towards integrating established laboratory methods with emerging technologies, aiming at the development of faster, more sensitive analytical systems applicable to quality control throughout the shrimp production chain.

Keywords: Crustaceans; sodium metabisulfite; sulfur-containing additive; analytical method; bibliometric analysis.

1. Introduction

Sulfites, or sulfiting agents, refer to a group of molecules that include sulfur dioxide (SO_2) and chemical congeners.[1,2] Within the food industry, sulfites are extensively utilized as food additives in a wide range of products, including dried fruits and vegetables, jams, flours, juices, and seafood, owing to their efficacy in inhibiting enzymatic browning reactions, oxidative degradation, enhancing shelf-life stability and exerting antimicrobial activity.[3,4]

Farmed shrimp production has seen significant growth worldwide in response to the growing demand for seafood. According to FAO,[5] between 2016 and 2020, shrimp farming registered an average annual growth of 8.99%. The pursuit of sustainable alternatives to capture fisheries, coupled with the increasing global demand for shrimp, has driven the expansion of shrimp aquaculture.

Consumption of fish products has increased in Brazil, reflecting a global trend due to their health benefits and increasing availability, reaching 9.5 kg/inhabitant/year.[6] However, per capita consumption is still below the recommendations of the World Health Organization (WHO) and the global average, which increased from 9.1 kg in 1961 to an estimated 20.7 kg in 2022.[7] Brazilian shrimp production under captive systems is predominantly concentrated in the Northeast region. Among the producing states, Ceará exhibits the highest productivity, with an output of 72,600 tons in 2023, corresponding to 57.02% of the national production.[8]

One of the problems in the shrimp processing industry is melanosis, a naturally occurring phenomenon in crustaceans resulting from complex biochemical reactions catalyzed by enzymes called polyphenol oxidases. These enzymes oxidize phenolic compounds present in crustacean tissues into dark pigments called melanin.[9] The intensity and rate of melanosis development in crustaceans are influenced by multiple factors, including storage time, temperature,

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physical damage, and species-specific characteristics. Crustaceans have a reduced shelf life due to melanosis, as it alters their sensory characteristics, drastically reducing the product's acceptability to consumers and, consequently, its market value.[10]

Among food additives, sodium metabisulfite ($Na_2S_2O_5$, INS 223) is one of the most extensively used chemical preservatives in food processing primarily due to its technological efficacy, ease of handling, and economic feasibility.[11] Its application is particularly prevalent in seafood processing, where it is employed to mitigate enzymatic melanosis, a post-harvest discoloration phenomenon commonly observed in shrimp.[12] However, despite their recognized advantages, substantial evidence has demonstrated that sulfite residues may pose health risks, especially when exposure exceeds established acceptable intake limits (e.g., 100 mg kg^{-1} in the edible part of the raw product or 30 mg kg^{-1} in the edible part of the cooked product), with respiratory effects being among the most frequently reported adverse outcomes.[5,13] Toxicological evidence indicates that excessive exposure to sulfites may cause irritation of the respiratory tract mucosa and increase the risk of gastrointestinal disturbances and asthma, particularly in susceptible individuals.[14,15]

The quantity of sulfites added to foods must achieve the desired objective without exceeding the maximum limits established by regulatory agencies.[16] The Codex Alimentarius classifies sulfites as one of the main allergens, with maximum limits of 10 mg kg^{-1} or 10 mg L^{-1} (as SO_2) whose presence must be marked on the product label.[17] In Europe, compatible concentrations of sulfite (expressed as SO_2) are between 50 and 300 mg kg^{-1} in raw and cooked shrimp and lobster. In Brazil and the USA, the maximum permitted concentration of sulfite is 100 mg kg^{-1} in raw shrimp and lobster and 30 mg kg^{-1} in cooked shrimp and lobster.[18]

The use of sulfites as food preservatives is well documented in the literature, and various detection techniques for different food matrices have been summarized.[17,19] Furthermore, recent research has addressed the occurrence, activity, toxicity, regulation, and detection methods of food additives, including sulfites.[20,21] However, articles regarding analytical methods for determining sulfites in shrimp remain scarce in the literature, despite advances in measuring sulfite at trace levels.

Nevertheless, there is a need to systematize knowledge regarding the main trends and analytical approaches used to determine sulfites in shrimp, given the lack of recent publications on this topic—as the last relevant studies appeared in the early 2000s. In light of this, the present study aims to analyze recent trends in analytical methods for determining sulfites in shrimp—focusing on electrochemical and chromatographic techniques—through a bibliometric analysis of scientific literature published between 2015 and 2024.

2. Experimental

2.1. Data collection and scope

A literature review was conducted using the Web of Science (WoS) database to collect scientific references on analytical methods for sulfite determination in shrimp published between 2015 and 2024. The VOSviewer® software version 1.6.20, supported by the Centre for Science and Technology Studies of Leiden University (The Netherlands), was used for the bibliometric investigation.[22]

2.2. Inclusion and exclusion criteria

The exclusion criteria used in the search were books, book chapters, and review articles. The area of knowledge was delimited to analytical chemistry, food technology, applied chemistry, and chemical engineering, to restrict the documents only to scientific research articles of interest. The titles and abstracts were required to include the terms used in the search keywords, in English: "sulfite," "food additive," "shrimp," and "validation methods." To obtain a more refined search on the topic, the logical operator "AND" was used to connect the parameters and the Boolean operator "OR" was used to combine synonyms.

3. Results and Discussion

3.1. Bibliometric analysis

The bibliometric search obtained after applying the exclusion filters resulted in identification of 539 articles published between 2015 and 2024 (Figure 1). The metadata set was extracted from the articles obtained, and a keyword co-occurrence network was generated using VOSviewer software. Thus, by extracting terms from the title and abstract of a document or from the list of keywords provided by the authors of a publication, a correlation network was established around the research theme. Figure 2 presents the bibliometric map, which identified 2,889 keywords with at least 5 occurrences. The size of the nodes (clusters) is proportional to the frequency of the author's keywords in the articles, and the links between the nodes, in turn, indicate the occurrence of the words.

The most relevant keyword was sulfite, as it was the subject of this research and is the most widely used additive in shrimp preservation. The use of sulfite in shrimp not only improves meat quality, preventing unwanted browning

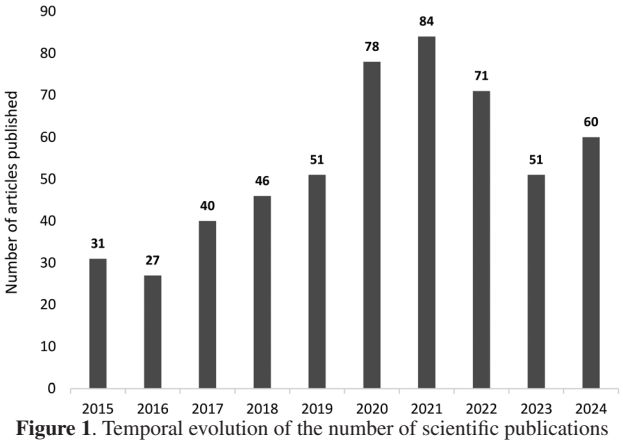


Figure 1. Temporal evolution of the number of scientific publications related to the determination of sulfites in shrimp from 2015 to 2024, based on data extracted from the Web of Science database

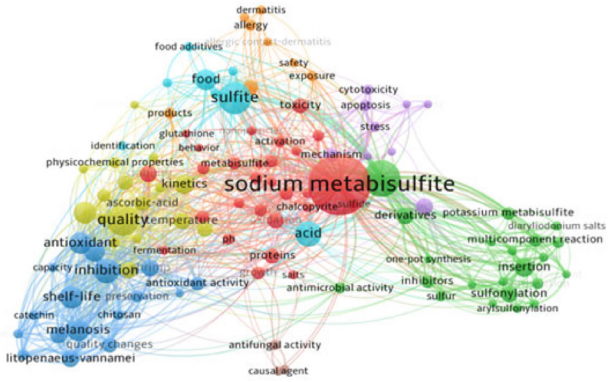


Figure 2. Co-occurrence map of keywords from publications related to methods for analyzing sulfites in shrimp

(melanosis), but can also extend the shelf life of the products.[23] Based on the keywords used in the searches, Table 1 presents the list of keywords that obtained the highest number of occurrences and the highest relevance. The most cited sulfite determination techniques were chromatography, electrochemical sensor, biosensor, and ion chromatography.

Analyzing the data in Figure 1, it can be observed that the number of publications on the use of sulfite in shrimp preservation during this period averaged 53.9 publications per year, win 30% of the publications occurring between 2020 and 2021. The database showed an increase in the number of published articles, showing a trend of annual growth in research, starting with 31 articles published in 2015, and reaching a peak of 84 articles published in 2021. This evolution indicates a growing interest from the scientific community in monitoring sulfite levels in shrimp, given the increased consumption of this crustacean, which has led to increased oversight by regulatory agencies regarding the use of sulfitizing agents, such as sodium metabisulfite, as it is a widely used preservative in fish preservation.

Table 1. Most frequent and relevant keyword

Keywords	Occurrence
Sulfite	100
Sulfur dioxide	71
Oxidation	50
Nanoparticles	25
Reduction	20
Chromatography	18
Electrochemical sensor	17
Biosensor	09
Ion Chromatography	08
Antioxidant activity	08

Regarding the co-occurrence of keywords (Figure 2), the clusters can be observed in a high-order Euclidean space dimension, allowing us to evaluate the conceptual structure of articles related to the use of sulfite in crustacean preservation, represented by the red cluster, which has the highest incidence, with 33 interconnected items. After refinement, 118 keywords remained that met this criterion. A co-occurrence network was generated with 118 keywords, 5 clusters, 780 links, and 1,358 edges. In the generated network, some words have greater visibility because they were cited more frequently in the articles found, such as: sulfite, SO_2, oxidation, degradation, and chromatography. The keywords "sulfite" and "SO_2" were the items identified with the highest occurrences and interactions. Five clusters with at least 15 keywords were formed. China is the world's largest shrimp producer, which explains the significant number of scientific publications related to the use of sodium metabisulfite in shrimp conservation. In numbers, this significant shrimp productivity represents 36% of global production, followed by India (8%), Indonesia (7%), Vietnam (5%), and Peru (3%). These five countries accounted for approximately 59% of global aquatic animal production in 2022.[7]

3.2. Use of sulfites to control melanosis

Melanosis, commonly known as "black spot," is a frequent problem in crustaceans during post-harvest storage; it compromises their sensory characteristics, thereby reducing quality, shelf life, and—consequently—commercial value.[24-25] The reaction is initiated by a naturally occurring enzyme, polyphenol oxidase (PPO)—also known as tyrosinase, polyphenolase, phenoloxidase, phenolase, cresolase, catecholase, and catechol oxidase—which acts as a catalyst for the darkening or black spotting of crustaceans. It is a crucial component of the arthropod proPO system and the key enzyme in melanin synthesis, the process responsible for this darkening.[24]

PPOs are distributed unevenly across different parts of the shrimp's body (Figure 3A), being located primarily in

the cuticle, muscle, and hemolymph.[10] Melanosis generally begins on the cephalothorax carapace, subsequently extending to the abdominal exoskeleton and the rest of the cephalothorax—particularly at the articulation zones between cuticle segments and the areas where the cuticle joins the pleopods—before spreading to the caudal region, including the telson and uropods.[25] Finally, melanosis is also influenced by species, sex, molting stage, capture method, and time of year.[10]

To prevent the occurrence of melanosis in shrimp, it is necessary to minimize PPO activity by controlling pH or temperature, as well as to use suitable PPO inhibitors, thereby preserving shrimp quality during handling, processing, and storage. Among the various options, sulfites are the most widely used agents following crustacean harvest.[26, 10]

Sulfites (INS 220–228)[27]—especially sodium metabisulfite (INS 223)—are the most effective and widely used additives for preventing melanosis in crustaceans (Figure 3B and 3C).[10,26,28] Immediately after capture, shrimp are exposed to sulfites via immersion, dusting, or spraying.[29] When sulfites are added to food (Figure 3D), an equilibrium is reached among three main forms of sulfur: sulfur dioxide (SO_2), bisulfite (HSO_3^-), and sulfite (SO_3^{2-}); the proportion of these species depends on pH, ionic strength, temperature, and concentration. When pH = pKa, 50% of the species are dissociated ($\alpha = 0.5$). Thus, at acidic pH (pH < 1.8), SO_2 predominates in the hydrated form $SO_2 \cdot H_2O$; in a slightly acidic medium (pH ~ 1.8 to 7.2), the bisulfite anion (HSO_3^-) predominates; and at neutral to alkaline pH (pH > 7.2), the predominant species is the sulfite anion (SO_3^{2-}).The undissociated form ($SO_2 \cdot H_2O$) exhibits the greatest antimicrobial potential due to its superior ability to diffuse across microbial cell membranes, allowing it to inhibit essential enzyme systems and metabolic processes.[1,17]

However, sulfites can cause adverse health effects[27,28] and induce a range of adverse clinical reactions, varying from dermatitis, urticaria, flushing, hypotension, abdominal pain, and diarrhea to life-threatening anaphylactic and asthmatic reactions.[30] Dermatological, gastrointestinal, and respiratory symptoms may occur in isolation or in combination, with respiratory symptoms being the most prevalent.[30,31] Furthermore, inhalation of high concentrations of SO_2 can cause acute toxicity affecting soft tissues and leading to respiratory distress, cyanosis, and death due to pulmonary edema.[24, 32]

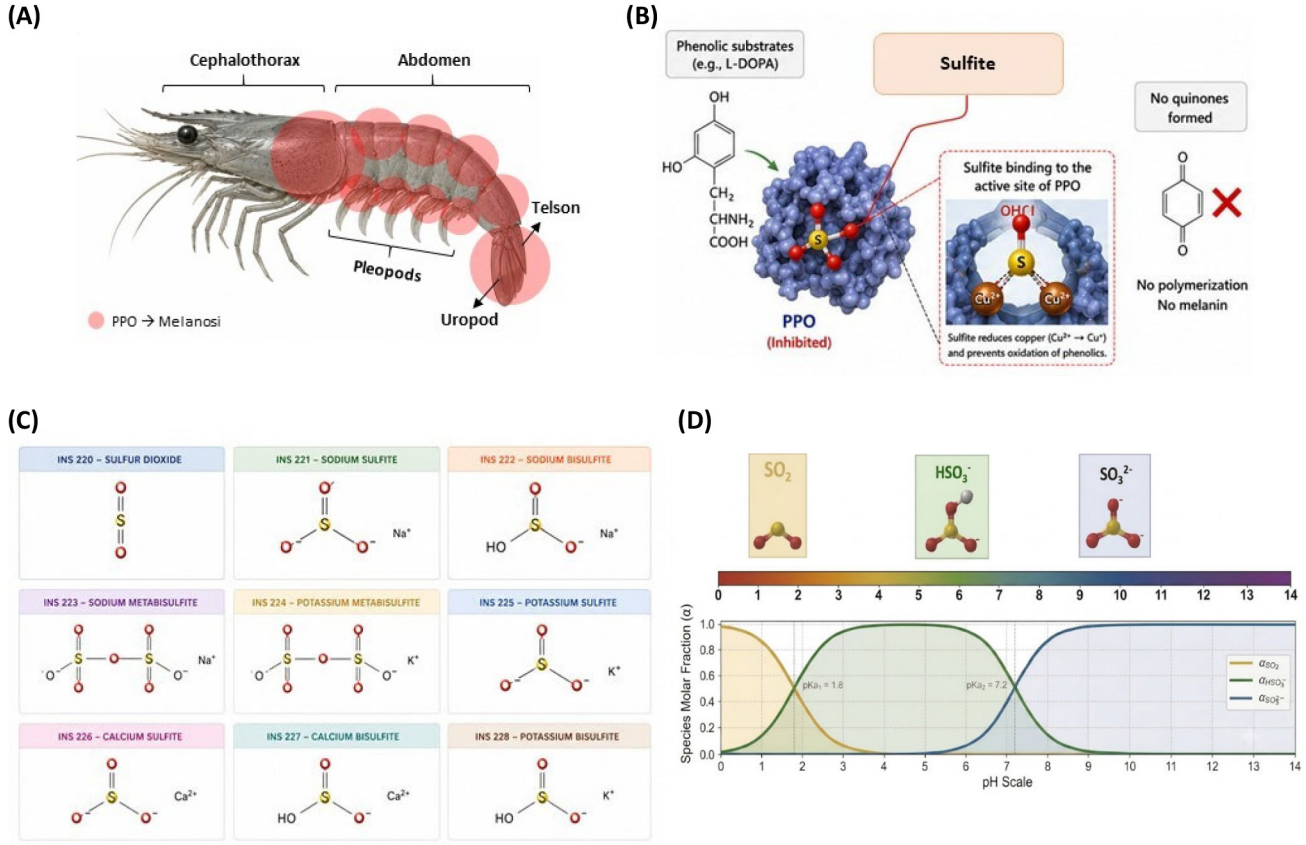


Figure 3. Mechanism of melanosis development and the role of sulfites in shrimp preservation. (A) Distribution of polyphenol oxidase (PPO) in shrimp tissues and the progression of melanosis after harvest; (B) Prevention of melanosis through the application of sulfiting agents, particularly sodium metabisulfite (INS 223); (C) Main sulfite application methods used during shrimp processing, including immersion, dusting, and spraying; (D) Chemical equilibrium of sulfite species in aqueous media ($SO_2 \cdot H_2O$, HSO_3^-, and SO_3^{2-}) as a function of pH, illustrating the mechanism responsible for antioxidant and antimicrobial activity. Source: the authors

3.3. Sulfite determination methods

For the identification and quantification of food additives present in raw materials or processed products, it is necessary to use analytical techniques to ensure quality control and food safety. Therefore, it is necessary to develop analytical techniques that provide reliability, selectivity and shorter analysis time.[33]

Due to the toxic effects that sulfite can have on public health, many efficient analytical methods—such as electrochemistry, spectrophotometry, enzymatic assays, chromatography, capillary electrophoresis, and titration—have been developed for the rapid and accurate quantification of this analyte in foods and beverages. Various analytical methods have been developed for the rapid and accurate quantification of this analyte in food, with electrochemical and chromatographic techniques being the most frequently cited for shrimp matrices.[34]

3.3.1. Monier-Williams method

The oldest known analytical method for sulfite quantification is the Monier-Williams method (Figure 4). This method, considered a reference, was first published in 1927 and is based on distillation followed by volumetric titration.[1] Although it is the official method recognized by the Association of Official Analytical Chemists (AOAC), the Monier-Williams method has certain disadvantages, such as the generation of large quantities of toxic solvents and a long quantification time—approximately 1.7 hours.[16]

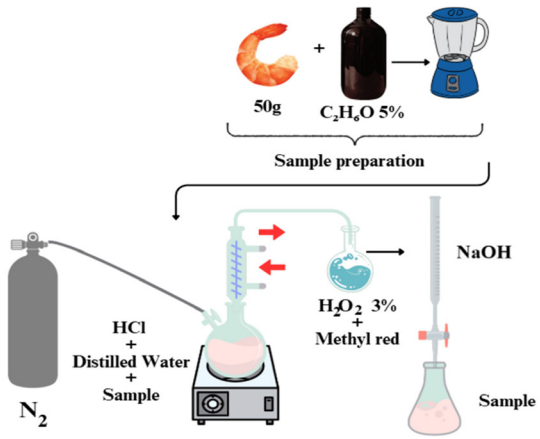


Figure 4. Monier-Williams system for sulfite determination. Source: the authors

This method consists of subjecting the sample to reflux in acid (0.5 M HCl) to release SO_2 (Equation 1), under a flow of nitrogen as the carrier gas bubbled through a 3% peroxide solution. The oxidation of the SO_2 gas to sulfate produces an acidic solution (Equation 2) which is subsequently titrated with standardized hydroxide and the initial sulfite concentration is estimated.

$$SO_3^{2-} + 2H^+ \rightarrow SO_2 \uparrow + H_2O \quad (1)$$

$$SO_2 + H_2O_2 \rightarrow SO_4^{2-} + 2H^+ \quad (2)$$

The procedure has the advantage of low cost and the need for few glassware and reagents. However, distillation is a time-consuming process and is unsuitable for determining low concentrations of sulfite. Several alternative methods to MW have been proposed, such as electrochemical,[35-38] flow injection analysis,[39] chromatographic,[40-43] capillary zone electrophoresis[18] and fluorescent sensors.[44]

3.3.2. Electrochemical

Electrochemical techniques, especially those based on ion-selective potentiometric electrodes, are recognized for their high selectivity, quantitative capability, rapid analytical performance, and high sensitivity, as well as their wide dynamic range and reduced response time simplicity.[45] Although other methods are applied for the determination and qualification of sulfite, electrochemical methods represent a promising alternative. Furthermore, electrode modification allows control of the morphology and size of the materials, enabling miniaturization and the development of portable electrochemical devices.[46] The redox properties of sulfite are well defined, allowing for its controlled oxidation or reduction, facilitating the modification and subsequent electrochemical determination of the analyte.[47]

Preparing shrimp samples for the electrochemical determination of sulfite requires rigorous strategies to overcome the analyte's high instability and the complexity of the biological matrix. To mitigate the rapid oxidation of sulfite to sulfate, muscle tissue homogenization must be performed at low temperatures with the addition of stabilizing agents. Furthermore, the strong tendency for electrode passivation caused by protein and fat adsorption necessitates efficient purification steps—including high-speed centrifugation, protein precipitation, and filtration through micrometric membranes—to yield a clear extract. Finally, depending on the analytical scope, either alkaline extraction (often using NaOH) is employed to quantify total matrix-bound sulfite, or rapid acid extraction is used for free sulfite; the resulting extract is then diluted in a supporting electrolyte with a strictly buffered pH to ensure the reproducibility and sensitivity of the voltammetric or amperometric measurements.[47]

Electrochemical systems typically consist of a three-electrode system, composed of a working electrode (WE), a reference electrode (RE), and an auxiliary or counter electrode (CE). Modifying the surface of the WE with different materials allows for the selective and specific detection of sulfite ions. These interface materials play a fundamental role in improving the selectivity, sensitivity, and stability of the electrochemical detection process of the

sulfite analyte. Various carbon materials, from traditional large-scale carbon materials (e.g., glassy carbon electrodes (GCEs), carbon paste electrodes (CPEs), and graphite) to well-known nanoscale carbon materials (e.g., carbon nanotubes (CNTs)), screen-printed electrodes (SPE), gold (Au) electrodes, and Pt electrodes, have wide use in electrochemical systems.[47]

Selective redox conversion of sulfite (SO_3^{2-}) to sulfate (SO_4^{2-}) can be achieved by enzymes, via biosensors.[47,48] Due to the specificity of the reaction, a high degree of selectivity can be achieved in the analytical method (Figure 5).

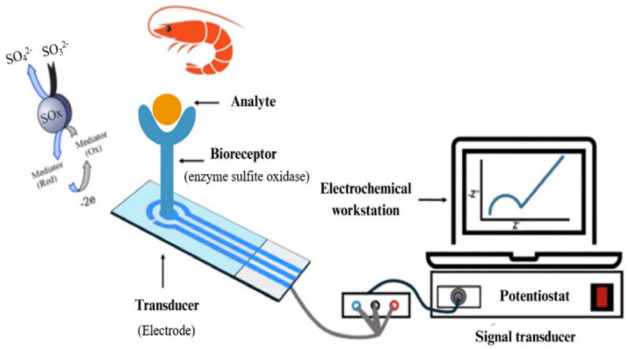


Figure 5. Main components of biosensors used for sulfite determination. Source: the authors

The biosensor is a device capable of providing an electrochemical signal proportional to the amount of sulfite present in the sample. The equipment consists of a compact benchtop potentiostat with an electrochemical cell integrated with disposable modified gold electrodes with specific redox enzymes. In short, an electric current is produced by the oxidation of the sulfite molecules present in the sample under study. When the sample containing sulfite is added, a redox reaction that involves the release of electrons with a variation of electric current proportional to the concentration added to the biosensor occurs. The equipment correlates the variation of the electric current with the sulfite value (mg kg^{-1}). This highly sensitive technique combines specificity and selectivity of the reactions.[17]

To develop a more efficient method for determining sulfite in shrimp, researchers developed the BIOFISH 300 SUL method, which consists of a highly specific biosensor based on its patented enzyme-based electrode for the rapid quantification of total sulfite.[17] The method consists of extracting sulfite from solid samples and subsequent quantification by the device in less than 3 minutes. The test kit consists of an electrochemical reader (BIOFISH 300 biosensor) and disposable electrodes (Biotest) that are capable of providing an electrical signal proportional to the amount of sulfite in the sample analyzed. The method proved to be accurate and presented good linearity, reproducibility and high sensitivity, as it was possible to quantify SO_2 at levels of 7 mg kg^{-1} with high precision in samples of raw and cooked shrimp.

Although different electrochemical methods for sulfite analysis in food have been reported in recent years, few studies have been applied to shrimp samples.[20,36] In general, electrochemical methods are limited by their strong dependence on the sample type, requiring adaptation or optimization of the instrumentation for their use in different food matrices. Another issue arises regarding the sensitivity of the electrochemical method, as contaminant incrustations may appear after a few analyses, rapidly decreasing the electrode sensitivity and requiring frequent reconditioning of the working electrode.

3.3.3. Chromatography

Chromatographic methods are widely used in the analysis of additives and contaminants in food, as they require a small number of samples and reduced analysis time, in addition to presenting good sensitivity and reliability of results. These methods are based on the separation of substances according to their affinity for two phases: a fixed phase, called the stationary phase, and a mobile phase, which flows over the fixed phase. Furthermore, it also allows for the identification and isolation of substances present in complex matrices such as food.[49]

Ion chromatography-IC (Figure 6), in turn, is defined as a technique based on the separation of ions present in a solution through their interactions with a stationary phase, usually composed of resins or columns containing functional groups, and a liquid mobile phase (ASTM E1151-93/2019). For ion detection, conductimetric and amperometric detectors are commonly used in the system.

Sample preparation of shrimp for sulfite determination via ion chromatography (IC) is a crucial step for efficiently extracting the analyte and converting it into a stable ionic form, while ensuring that matrix macromolecules do not damage the column's anion-exchange resin. The most common strategy—recommended by regulatory agencies—involves rapid acid distillation (a modification of the Monier-Williams method), which oxidizes sulfite to sulfate, a highly stable species ideal for chromatographic separation by elution. For direct extraction methods that do not involve distillation, alkaline extraction solutions or formaldehyde buffers are used to stabilize the sulfite as a formaldehyde-sulfite adduct; these steps must be followed by high-speed centrifugation, rigorous protein precipitation, and filtration through solid-phase extraction (SPE) cartridges or 0.22 µm membranes to remove organic interferents and excess chloride ions, thereby preventing column overload and loss of resolution in the ion-exchange column.[50]

Several studies have cited the use of ion chromatography with conductivity detection for the analysis of sulfites in

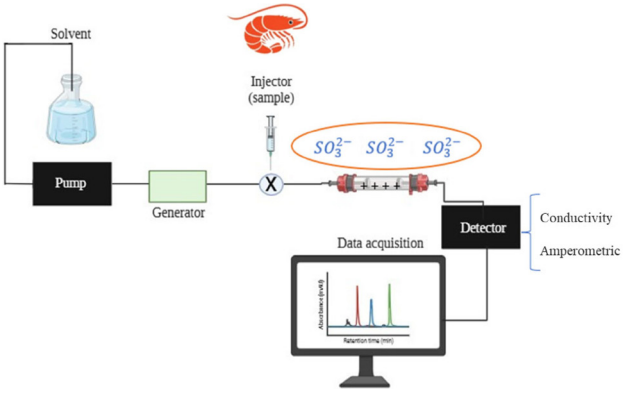


Figure 6. Main components of ion chromatography system. Source: the authors

food.[42,50,51] Berardi *et al.*[50] used the ion chromatography method with suppressed conductivity detection in a study on the effect of different cooking methods (grilled, roasted, fried, steamed, and baked) on shrimp samples, in order to verify the effect of cooking on the reduction of residual sulfite. For sample extraction, the authors used an alkaline stabilizing solution (50 mM NaOH and 10 mM fructose) in ultrapure water to retard the oxidation of sulfite to sulfate and remove certain interfering matrix compounds. The method was validated and proved to be efficient in terms of linearity, sensitivity, selectivity, precision, robustness, and measurement uncertainty. The study concluded that cooking reduces sulfite levels in shrimp samples, with steaming showing the greatest reduction (55.3%), while baking showed the least reduction (13.9%).

To quantify residual sulfite levels in Mediterranean red shrimp, Mammina *et al.*[52] also used the IC method. The shrimp samples were subjected to acid distillation, and the resulting product was purified and injected into an ion chromatograph using Na_2CO_3 as the eluent. The method was validated and proved to be efficient, accurate, and require less analysis time compared to conventional methods. In the study in question, of the 100 samples analyzed, 0.7% showed levels below the detection limit, 12.7% showed levels above the legal limit, and in the remainder, the quantified levels were below the maximum allowed by law.

Other chromatographic techniques have also been reported for the analysis of sulfur-containing additives in shrimp.[14,53] Mu *et al.*[14] quantified SO_2 in food by high-performance liquid chromatography with a high-sensitivity fluorescence detector, allowing the quantification of low levels of SO_2. According to the validation, the method showed good linearity (R^2 = 0.996), with a recovery of 80-110%, demonstrating its accuracy, with LOD = 0.2 mg/kg and LOQ = 0.7 mg/kg. In this study, several food samples were analyzed. Yang *et al.*[53] quantified the levels of free and total sulfites in shrimp by high-performance liquid chromatography using pre-column derivatization with 2,2'-dithiobis(5-nitropyridine), a procedure whose selectivity was confirmed by LC-MS data, followed by UV detection. The method showed a linear range of 3.2 to 51.2 mg L^{-1}, with a detection limit of 0.3 mg L^{-1}. In frozen shrimp samples, the determined levels of free and total sulfites were 26.58 ± 0.48 mg kg^{-1} and 31.44 ± 0.83 mg kg^{-1}, respectively. According to the results obtained, the method proved suitable for quantifying the residual content of sulfites in food.

3.4. Comparison and analysis of sulfite detection methods in shrimp

The choice of the appropriate method for sulfite determination will depend on the sample to be analyzed, its preparation, and the objective of the analysis. More modern techniques described are indicated because they require a minimal amount of sample and a short analysis time. The comparative assessment of the analytical methods summarized in Table 2 demonstrates that sulfite determination in shrimp remains a challenging task because of the intrinsic complexity of the sample matrix. Shrimp muscle is characterized by high moisture content, elevated protein concentration, endogenous sulfur-containing compounds, and intense post-mortem biochemical activity.[12,54,55] These characteristics may affect sulfite stability through oxidation, volatilization, or binding reactions with carbonyl compounds and proteins, making accurate quantification highly dependent on sample preparation and extraction efficiency.[12,17]

Among the available methodologies, the Monier–Williams method continues to be recognized as the international reference procedure for regulatory purposes due to its standardized protocol and broad acceptance by official agencies.[16] Nevertheless, this technique presents significant operational limitations, including long analysis times, multiple sample handling steps, acid distillation requirements, and susceptibility to positive bias caused by naturally occurring sulfur compounds in seafood matrices.[40] These drawbacks reduce its suitability for routine industrial monitoring, where rapid analytical response is essential for production decision-making.

Chromatographic techniques, particularly ion chromatography (IC) and high-performance liquid chromatography (HPLC), currently represent the most reliable laboratory-based approaches for sulfite determination in shrimp. Their superior selectivity and sensitivity enable accurate quantification even in complex biological matrices, while minimizing analytical interference. Furthermore, these methods provide excellent precision and reproducibility, making them suitable for confirmatory analysis and regulatory surveillance.[42,50,52]

Table 2. Analytical methods reported for the analysis of sulfite additives in shrimp

Method	Analytical Principle	LOD[e] (mg kg^{-1})	Analysis Time	Advantages	Limitations	Applications	References
Monier–Williams	Acid distillation followed by SO_2 collection and titration	10–20	60–120 min	Official reference method, internationally accepted, suitable for regulatory compliance	Labor-intensive; time-consuming; possible matrix interferences; limited sensitivity for trace levels	Regulatory and confirmatory analysis	41,56
FIA[a]	Spectrophotometric detection after chemical reaction	2–10	<10 min	High sample throughput, automation, reduced reagent consumption	Lower selectivity; matrix-dependent performance	Research applications and Laboratory analysis	39
IC[b]	Separation of sulfite ions with conductivity detection	<1	15–30 min	Excellent selectivity and accuracy, suitable for complex matrices, low detection limits	Expensive instrumentation; skilled operator required	Research applications and Laboratory analysis	50, 52,53
HPLC[c]	Chromatographic separation with UV or fluorescence detection	<1	20–40 min	High sensitivity and reproducibility, excellent quantitative performance	Derivatization may be required; relatively high operational cost	Research applications and Laboratory analysis	14,16,53
CZE[d]	Separation according to electrophoretic mobility	1–5	10–20 min	High separation efficiency, low solvent consumption	Limited routine application; specialized instrumentation	Research applications and Laboratory analysis	18,56,57
Electrochemical Sensors	Electrochemical oxidation/reduction of sulfite	0.1–5	<5 min	Rapid response, portable, low reagent consumption, suitable for field analysis	Electrode fouling, interference from electroactive compounds, calibration required	On-site monitoring, rapid quality control and research applications	35-37, 47
Biosensors	Enzyme-catalyzed sulfite oxidation coupled with amperometric detection	0.05-2	2–5 min	High selectivity, excellent sensitivity, suitable for real-time analysis	Limited enzyme stability, sensor lifespan, higher fabrication cost	Industrial quality control, rapid screening and research applications	17,48,58

[a] FIA: Flow Injection Analysis; [b] IC: ion chromatography; [c] HPLC: high-performance liquid chromatography; [d] CZE: capillary zone electrophoresis; [e] LOD: Limit of Detection.

However, widespread implementation is often constrained by high instrumentation costs, extensive sample preparation procedures, and the need for highly trained personnel.

In contrast, electrochemical methods have emerged as one of the fastest-growing research areas identified in the bibliometric analysis. Advances in nanostructured electrode materials, conductive polymers, carbon nanomaterials, and metal nanoparticles have substantially improved analytical sensitivity, electron transfer efficiency, and detection limits.[35-37,47] Their low cost, rapid response, portability, and compatibility with miniaturized devices make electrochemical sensors particularly attractive for on-site applications throughout the shrimp production chain.

Despite these advantages, electrochemical methods still face important challenges before large-scale industrial implementation can be achieved. Matrix effects caused by proteins, salts, lipids, and other electroactive compounds frequently lead to electrode fouling, signal instability, and reduced long-term reproducibility.[59,60] Consequently, improvements in electrode surface engineering, antifouling materials, and sample pretreatment strategies remain essential research priorities.

Biosensors constitute an important evolution of this technology by combining the specificity of biological recognition elements with the sensitivity of electrochemical transducers. The incorporation of sulfite oxidase and other enzymatic systems has significantly enhanced analytical selectivity while reducing interference from complex food matrices.[16,48] In addition, recent biosensor platforms have demonstrated rapid response times, low limits of

detection, and the potential for real-time monitoring during shrimp processing. Nevertheless, enzyme stability, storage conditions, fabrication cost, and interlaboratory validation continue to represent significant barriers to commercial deployment.

An important finding arising from this review is that the majority of published studies focus primarily on analytical performance parameters—such as limit of detection, linearity, precision, and recovery—whereas data on the method's robustness and feasibility under real industrial conditions are scarce. Variables including species differences, farming systems, storage duration, freezing–thawing cycles, cooking processes, and commercial handling practices can significantly influence residual sulfite concentrations and, consequently, analytical accuracy. This gap highlights the need for validation studies using naturally incurred commercial samples rather than laboratory-spiked matrices.[16,50]

The bibliometric analysis also indicates a clear shift in research priorities over the last decade. Earlier studies were predominantly devoted to improving conventional laboratory techniques, whereas more recent publications increasingly emphasize portable sensors, nanotechnology-based analytical platforms, biosensors, and point-of-care devices. This transition reflects the growing demand for rapid, cost-effective, and decentralized analytical tools capable of supporting Hazard Analysis and Critical Control Point (HACCP) programs, traceability systems, and regulatory inspection throughout the seafood supply chain.[61,62] Figure 7 presents a two-dimensional comparative analysis correlating analytical efficiency (determined by response speed and sensitivity/low limit of detection) with estimated costs (instrumental and operational) for sulfite detection methods.

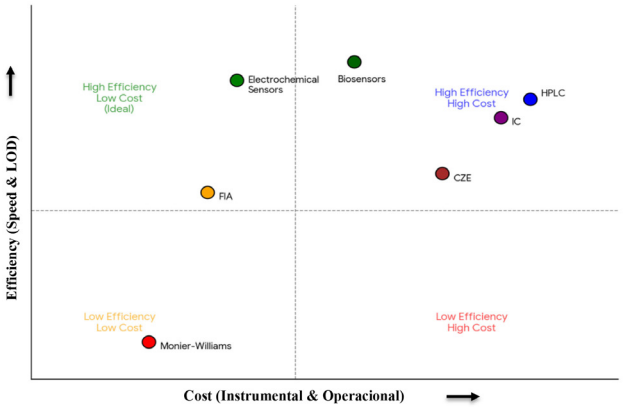


Figure 7. Comparative analysis of efficiency versus costs for analytical methods to determine sulfites in shrimp. Source: the authors

Overall, the current evidence suggests that no single analytical technique simultaneously fulfills all requirements regarding sensitivity, selectivity, operational simplicity, cost-effectiveness, and regulatory acceptance. Chromatographic methods remain indispensable as reference techniques for confirmatory analysis, whereas electrochemical sensors and biosensors are becoming increasingly promising alternatives for rapid screening and real-time monitoring. Future research should therefore prioritize method standardization, interlaboratory validation, assessment of matrix effects across different shrimp species, and integration of portable analytical technologies into industrial quality-control programs. Such developments will contribute to more reliable sulfite monitoring, improved consumer protection, and greater compliance with international food safety regulations.

4. Conclusion

The mini-review presented revealed numerous studies addressing different analytical techniques for the determination of sulfites in food. The bibliometric analysis revealed significant growth in publications over the last decade, accompanied by a diversification of the analytical approaches employed. The results indicate progress in the development of various sulfite detection methods, reflecting the quest for analytical systems that are faster, more sensitive, and compatible with field applications and real-time monitoring. However, for the analysis of sulfites in shrimp samples, publications have been more limited.

A comparison of available methods demonstrated that no single technique can simultaneously meet the requirements for sensitivity, selectivity, speed, and low cost. The Monier–Williams method remains the official reference for regulatory purposes, while ion chromatography and HPLC continue to be the most robust techniques for confirmatory analysis due to their high precision and reliability. On the other hand, electrochemical sensors and biosensors represent the most promising technologies for on-site applications, although they still require advancements regarding stability, standardization, and inter-laboratory validation. Another relevant aspect identified in this review is that most studies focus on evaluating analytical performance under controlled laboratory conditions; there is a scarcity of research considering the variability inherent in different shrimp species, farming systems, storage conditions, and industrial processing stages. This gap limits the transfer of results to large-scale applications and highlights the need for studies that validate methodologies under conditions representative of the production chain. The study reinforces the need for the implementation of innovative and modern analytical techniques for the analysis of sulfites in shrimp samples, to ensure compliance with international regulations and food safety.

Acknowledgements

The authors would like to thank FUNCAP (BP6-0241-00060.01.00/25), CNPqUniversal (406655/2023-5), CNPq INCT-ALIM (406760/2022-5) and CAPES PRAPG (88887.909618/2023-00) for the financial support of this research and IFCE and NUTEC for the scientific support.

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